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Review

Activation of the Dorsal Raphe Nucleus and Locus Coeruleus by Transcutaneous Electrical Nerve Stimulation in Alzheimer's Disease: A Reconsideration of Stimulation-Parameters Derived from Animal Studies

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Abstract

In 1990 a series of studies started in which the effects of Transcutaneous Electrical Nerve Stimulation (TENS) was examined on cognition, behaviour, and the rest-activity rhythm of patients with Alzheimer's disease (AD). In these studies, TENS aimed primarily at stimulating the dorsal raphe nucleus (DRN) and the locus coeruleus (LC) by a combination of low- and high-frequency stimulation (2 Hz and 160 Hz, respectively), a pulse width of 0.1 ms, and an intensity that provokes muscular twitches. TENS was applied 30 min a day, during a six-week period. In order to make reliable comparisons between studies, identical stimulation-parameters were used in all studies thus far. TENS appeared to have a positive effect on cognition, behaviour, and the rest-activity rhythm but the effects disappeared after cessation of stimulation. In order to optimise TENS treatment in AD, the present paper is meant to reconsider the once selected stimulation-parameters by reviewing the relevant literature published since 1991. The results derived from animal experimental studies show that for an optimal stimulation of the LC and DRN, the pulse width should be more than 0.1 ms. Limitations and suggestions for future research will be discussed.

Key Words: dorsal raphe nucleus, locus coeruleus, transcutaneous electrical nerve stimulation, Alzheimer's disease

Alzheimer's Disease

Epidemiology

At the age of 65, the prevalence of dementia is about 1.5% and increases to about 30% at the age of 80 (33). Within dementia, Alzheimer's disease (AD) is the most common cause, affecting 60% to 70% of all cognitively impaired elderly. The number of AD patients has been estimated at 2.3 million in the USA (7). The number of new cases of AD each year (incidence) is approximately 360.000, implying 40 new cases each hour (7). Besides problems for the individual patients and their surroundings, the increasing proportion of elderly people in most countries will cause great burden to health care systems and economy in the near future.

Since there is no cure for AD at this moment, research on (non-)pharmacological interventions that may stabilize or even improve the clinical course of the disease is crucial.

Neuropathology in AD

AD is characterized by a progressive neuropathology in the temporo-parietal, frontal and occipital lobes (6). More specifically, the hippocampus, which plays a crucial role in memory (27), is affected (3,35),

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even in a pre-clinical stage (16). The prefrontal cortex, which is involved in executive functions like cognitive flexibility, planning, and response inhibition (10) also degenerates in AD (6). Furthermore, the hypothalamus and particularly the hypothalamic suprachiasmatic nucleus (SCN), involved in affective behaviour and the regulation of the circadian rest-activity rhythm, respectively (45, 46, 51), show neuropathological changes in AD (45, 46). Importantly, nightly restlessness is often the main reason for institutionalisation (32).

'use it or lose it'

It is noteworthy that the neuropathological hallmark of AD is not cell death but atrophy (47). Shrunken cells that still have some metabolism characterize brain atrophy. Swaab and colleagues (47) provide convincing evidence that the decreased metabolism in AD can be enhanced by the reactivation of shrunken cells. This reactivation may result from neuronal stimulation that subsequently slows down or even restores degenerative processes, a hypothesis that has been paraphrased as 'use it or lose it' (44). In other words, despite the severe neuropathology in cortical and subcortical areas, suppressing clinical symptoms and positively influencing the course of this progressive disease by neuronal stimulation is still possible. Neuronal stimulation could take place by various types of pharmacological and, interestingly, also by non-pharmacological stimuli. An example of a non-pharmacological treatment strategy that might enhance the decreased metabolism in AD is Transcutaneous Electrical Nerve Stimulation (TENS).

Transcutaneous Electrical Nerve Stimulation (TENS) in AD

In the preceding twelve years, TENS has been widely studied in AD patients (37,39-43,50). In all but one study, a 30 min-a-day and 5 days-a-week TENS treatment was applied to patients in a relatively early stage of AD, based on the assumption that the earlier the intervention, the more effective. Each study also included a control group that received sham stimulation. Neuropsychological functions, behaviour and rest-activity rhythm were assessed at three moments, i.e. before and directly after the six-weeks treatment period and again after a treatment-free period of six weeks.

Except for the first pilot-study (37), the stimulation-parameters used were exactly the same in all studies. The TENS-device (Premier 10 s) generated two 'bursts' a second (2 Hz) of biphasic impulses with an internal frequency of 160 Hz (BURST-TENS) (11). The pulse width was 0.1 msec. Based on animal experimental studies (4,5) available at that time, it was argued that low-frequency TENS (2 Hz) comprising high intensity 0.1 ms spikes could activate the locus coeruleus (LC) whereas high-frequency (>10 Hz) stimulation comprising the same pulse width and intensity increased the activity of the dorsal raphe nucleus (DRN). The DRN and LC are the origin of the ascending serotonergic and noradrenergic neurotransmitter systems, respectively (22), and show neuronal loss in early stage AD (29). Importantly, studies have shown afferent and efferent pathways that connect the DRN and LC with the hypothalamus, and specifically with the SCN (4, 21, 24, 53), the septal/hippocampal region (14, 15, 26, 53), and the frontal lobe (20, 53).

Results of the TENS-studies show that, in comparison with a placebo treatment, TENS improved nonverbal short-term memory, nonverbal and verbal long-term recognition memory, and executive functioning (verbal fluency) in AD patients. Moreover, TENS had a positive effect on affective behaviour, e. g. depressive symptoms declined. Another important finding was that nightly restlessness decreased in TENS-treated patients.

Notably, in all studies, after cessation of stimulation the observed improvements disappeared. In order to optimise TENS treatment in AD, e.g. maintaining positive effects after ending the treatment, the present paper reconsiders the once selected stimulation-parameters to stimulate primarily the DRN and LC, by reviewing relevant literature published since 1991. All reviewed studies are animal experimental studies. A frequency of < 10 Hz will be considered *low* and a frequency of ≥ 10 Hz will be considered *high* (38).

The present paper will first focus on indirect electrical stimulation, i.e. stimulation through the peripheral nervous system, of the DRN/serotonergic system and the LC/noradrenergic system. Subsequently, studies on direct stimulation of both brain stem nuclei and its effect on supraspinal areas, particularly the hippocampus, the hypothalamus including the hypothalamic SCN, and the prefrontal cortex will be presented. Limitations and suggestions for future research are discussed.

Indirect DRN and LC Stimulation

Increased activity of the DRN, measured by c-fos protein expression, has been observed after low-frequency (3 Hz) electro-acupuncture (EA) of Zusanli (St 36) in the hind leg of rats (8). The intensity was high enough (20 V) to provoke slight muscular twitches of the hind limb. The pulse-width was 10 ms and the duration of EA was 1 hour. An increase in activation of the LC-hypothalamic pathway of aged

Table 1. Indirect stimulation of the dorsal raphe nucleus (DRN) and the locus coeruleus (LC). na: not available.

Indirect DRN stimulation	Pulse form	Pulse width	Intensity	Frequency	Duration of stimulation	Results
Dai & Zhu, 1992	na	10 ms	20 V	3 Hz	1 hour	Increased activity DRN
Kwon *et al*., 2000	Biphasic impulses	0.5 ms	6 V	4 Hz and 100 Hz	2 hours	Increased activity DRN, particularly with 100 Hz
Indirect LC stimulation						
Rouzade-Dominquez et al., 2001	na	0.5 ms	1.3 mA	0.1 Hz	trial of 60 pulses	Increased activity LC
Zhu *et al*., 2000	Continuous wave	na	1-3 V	4 Hz	3 minutes	Increased activity in the brain stem-hypothalamus pathway
Kwon *et al*., 2000	Biphasic impulses	0.5 ms	6 V	4 Hz and 100 Hz	2 hours	Increased activity LC, particularly with 4 Hz

rats has been observed after EA of Shenshu (UB23) (56). Stimulation parameters were: a frequency of 4 Hz, an intensity of 1-3 V, a continuous wave pulse form, and a duration of stimulation of 3 minutes. No information on the pulse width was provided. The LC-noradrenergic neurons of rats could also be activated by sciatic nerve stimulation with a frequency of 0.1 Hz, a pulse width of 0.5 ms, and an intensity of 1.3 mA (34). The trial consisted of 60 pulses. Again, no information on the waveform was provided.

In one study, activation of both the DRN and LC was measured (25). Zusanli was stimulated with a frequency of 4 Hz or 100 Hz and a pulse width of 0.5 ms. An intensity of 5 times threshold (mean value 6 V) provoked a muscle twitch. Duration of stimulation was 2 hours and the pulsform was biphasic. Results show that low-frequency stimulation of 4 Hz had a larger effect on the LC compared to the DRN. However, both brain stem areas were equally activated by high-frequency stimulation of 100 Hz.

Taken together, although both brain stem nuclei respond to low- and high-frequency indirect stimulation, the LC reacts somewhat stronger to low-frequency stimulation than the DRN. The various stimulation-parameters and concurrent results are presented in Table 1.

Direct DRN and LC Stimulation

Direct DRN Stimulation: the Hippocampus

Ezrokhi and co-workers (12) observed that direct high-frequency stimulation of the DRN of rats had a beneficial influence on the long-term potentiation (LTP) decay at the synapses of the hippocampus. LTP implies an activity-dependent increase in synaptic transmission efficiency that may last for hours and represents the mechanism underlying conscious memory (2). In the study of Ezrokhi et al. (12), the stimulation parameters were: a frequency of 100 Hz, a pulse width of 0.4 ms, and an intensity between ±100 - 400 μA. Biphasic square constant current pulses were used and the duration of stimulation varied from hours to days. Unfortunately, there was no further information on which intensity was the most effective.

In another study, the effects of direct DRN stimulation on various brain areas were examined (31). The amount of 5-hydroxytryptamine (5-HT) increased in, among others, the ventral hippocampus and the medial septum. No effect was observed in the dorsal hippocampus. Stimulation-parameters were: a frequency of 5 Hz, an intensity of 300 μA and 1 ms pulse width. Duration of stimulation was 20 min. The waveform was not mentioned. In an earlier study, McQuade and Sharp (30) applied the same intensity and pulse width in four different frequencies, i.e. 2, 3, 5 and 10 Hz. The results show that the higher the frequency, the more release of 5-HT in the hippocampus of the anaesthetized rat.

Direct DRN Stimulation: the Hypothalamus

Activity of several hypothalamic nuclei can be enhanced by electrical stimulation of the DRN neurons. Saphier (36) observed that subgroups of neurons in the hypothalamic paraventricular nucleus (PVN) of rats responded differently to DRN stimulation. The PVN plays a role in autonomic and neuroendocrine processes (45,48). Direct stimulation of the

Table 2. Direct dorsal raphe nucleus (DRN) stimulation and its effect on the hippocampus, hypothalamus and prefrontal cortex. PVN: Paraventricular nucleus; 5-HT: 5- hydroxytryptamine; LTP: long-term potentiation. na: not available.

	Pulse form	Pulse width	Intensity	Frequency	Duration of stimulation	Results
Ezrokhi *et al*., 1999	Biphasic square - wave	0.4 ms	±100-400 μA	100 Hz	1 minute	Restoration of a decay in LTP in CA1
McQuade & Sharp, 1997	na	1 ms	300 μA	5 Hz	20 minutes	Release of 5-HT in ventral hippocampus and medial septum
McQuade & Sharp, 1995	na	1 ms	300 μA	2,3,5,10 Hz	20 minutes	The higher the frequency, the more the release of 5-HT in the hippocampus
Saphier, 1991	Bipolar square - wave	1 ms	1000 μA	0.2 - 0.5 Hz	na	Excitation (53%) and inhibition (13%) of PVN neurons
Weidenfeld *et al*., 2002	na	1 ms	500 μA	100 Hz	5 minutes	Increase in the hypothalamic PVN extracellular 5-HT levels
Gartside *et al*., 2000	Square-wave	1 ms	300 μA	3 Hz: single or twin-pulses	10 minutes	Release of 5-HT in the medial prefrontal cortex. Twin two-times more than single

DRN took place at a frequency of 0.2 Hz - 0.5 Hz, a pulse width of 1 ms and an intensity of 1 mA. The pulse form was a bipolar square-wave. Eight out of 15 neurons were activated (53%), two cells showed an inhibition (13%) whereas four cells (33%) did not respond at all. Considering the latency of the response after stimulation of the DRN, a monosynaptic pathway between the DRN and the PVN is suggested (36).

Interestingly, Weidenfeld and colleagues (54) observed in a recent animal experimental study that, by DRN stimulation, the PVN showed an increase in its extracellular release of 5-HT, with a subsequent activation of the hypothalamus-pituitary-adreno-cortical (HPA) axis. The stimulation-parameters were: a frequency of 100 Hz, a pulse width of 1 ms, an intensity of 0.5 mA, and duration of stimulation of 5 min. No information about the waveform was available.

Direct DRN Stimulation: the Frontal Lobe

Gartside and colleagues (17) compared single-pulse stimulation with twin-pulse stimulation (burst firing) of the DRN with respect to the release of 5-HT in the medial prefrontal cortex of rats. Stimulation parameters included a frequency of 3 Hz, a pulse width of 1 ms and an intensity of 300 μA. The pulse-form was a square-wave and duration of stimulation was 10 min. The stimuli were applied singly or in pairs with an interval of 10 ms between the pulses (burst firing). The results show that, compared to the single pulses, the twin pulses doubled the release of serotonin in the medial prefrontal cortex.

In sum, literature points to a positive relationship between the frequency of DRN stimulation and release of 5-HT in adjacent brain areas. The utilization of a burst signal resulted in a higher release of 5-HT in the medial prefrontal cortex (for details, see Table 2).

Direct LC Stimulation: the Hippocampus

In the study of Ezroki and colleagues (12), it was observed that decay in LTP at synapses of the perforant pathway and dentate gyrus of the hippocampal formation could be restored by high-frequency (50-100 Hz) stimulation of the LC. Other stimulation parameters were: a pulse width of 0.1 - 0.4 ms, an intensity of 65 - 300 μA, and a biphasic square constant current waveform. The study design included variability in stimulation frequency, pulse width and intensity but information about the most effective combination of these three parameters is lacking. The

Table 3. Direct locus coeruleus (LC) stimulation and its effect on the hippocampus, hypothalamus and prefrontal cortex. LTP: long-term potentiation; PVN: paraventricular nucleus (hypothalamus). na: not available.

	Pulse form	Pulse width	Intensity	Frequency	Duration of stimulation	Results
Ezrokhi *et al.*, 1999	Biphasic square constant current	0.1 - 0.4 ms	65 - 300 μA	50-100 Hz, one to two trains: 15-20 seconds interval	1 minute	Restoration of a decay in LTP in perforant pathway and dentate gyrus
Velley *et al.*, 1991	Sinusoidal waveform	0.2 ms	60 μA	100 Hz	15 minutes	Increase in α_2-receptor sites density in the hypo thalamus
Lookingland *et al.*, 1991	Monophasic pulses	1 ms	400 μA	15 Hz	10 minutes	Increase in the noradrenergic metabolite 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) in the PVN
Florin-Lechner *et al.*, 1996	na	0.2 ms	700 μA	Tonic: 3,5,10 Hz	20 minutes	The higher the frequency, the higher the norepinephrine increase in perfrontal cortex
				Bursts of 6 Hz Bursts of 12 Hz Bursts of 24 Hz		Bursts more effective than tonic. Highest increase in norepinephrine at 12 Hz

duration of stimulation was 1 min.

It has been suggested that both LTP and short-term potentiation of the perforant-path in the awake rat results from a phasic instead of tonic LC cell firing (23). Interestingly, particularly non-noxious stimuli yield a phasic activation of LC cells (1).

Direct LC Stimulation: the Hypothalamus

LC-stimulation with 100 Hz, pulse width of 0.2 ms, a sinusoidal waveform, and an intensity of 60 μA, increased the density of α_2-receptor sites in the hypothalamus of rats (52). The authors argue that this sequence of events causes a reduction in stress reaction and hence improves cognitive functioning. In another animal experimental study, direct LC stimulation increased the activity in the hypothalamic paraventricular nucleus (PVN), reflected in an increase in the noradrenergic metabolite 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) (28). Electrical stimulation of the LC took place by monophasic pulses, with a pulse width of 1 ms, an intensity of 400 μA, and a frequency of 15 Hz.

Direct LC Stimulation: the Frontal lobe

Florin-Lechner and colleagues (13) stimulated the LC of rats either with tonic stimulation (evenly spaced pulses) or with phasic stimulation (bursts of pulses). In the tonic stimulation condition 3, 5, or 10 Hz was used for 20 minutes. The results showed a frequency-dependent release of norepinephrine in the prefrontal cortex, i.e. the higher the frequency, the higher the release. Interestingly, compared to the 3 Hz tonic stimulation, bursts of 3 pulses (presented at 6, 12, and 24 Hz, every second) produced a much larger increase in norepinephrine, with the largest increase at 12 Hz. It is concluded that the physiological relevant 'burst' activity of LC neurons releases norepinephrine in the prefrontal cortex in the most effective way.

In sum, similar to the DRN, frequency of direct LC-stimulation and release of norepinephrine in associated areas shows a positive relationship. A summation of the effects of the various stimulation-parameters is presented in Table 3.

Discussion

The goal of the present study is to examine whether studies on the effects of indirect and direct stimulation of the DRN and LC published from 1991 until now still support the originally selected stimulation-parameters that were used in our TENS-studies. In those TENS-studies, it was argued that low-frequency TENS (2 Hz) with a pulse width of 0. 1 ms, could stimulate the LC whereas high-frequency stimulation of 160 Hz, in combination with the same pulse width, could increase the activity of the DRN. Except for the pilot-study (37), the intensity of the TENS signal used provoked muscular twitches. Both frequencies were combined into one TENS-mode, i.e. BURST-TENS (11).

Frequency

The results of the present review indicate that the LC, compared to the DRN, responds more strongly to indirect *low-frequency* stimulation, i.e. < 10 Hz. In addition, direct *high-frequency* stimulation of the LC with frequencies varying from 10 Hz to 100 Hz resulted in the highest activity increase in the hippocampus, the hypothalamus, and the prefrontal cortex. With respect to the DRN, the results of both direct and indirect stimulation studies show that this brain stem nucleus preferably responds to *high-frequency* stimulation of 10 Hz, 20 Hz and 100 Hz.

The finding that both the LC and the DRN respond positively to a burst-firing rate is not so surprising considering the electrophysiological characteristics of the neurons of the DRN and LC. It has been observed that a considerable number of DRN and LC neurons are capable of firing in bursts (17-19, 55).

Pulse Width

As mentioned before, the pulse width used in our TENS-studies was 0.1 ms. (43). Although in the here reviewed direct and indirect stimulation studies the pulse width varied between 0.1 ms - 10 ms, the most frequently applied pulse widths were 0.4/0.5 ms and 1 ms. Future research is necessary to find out whether an increase in pulse width is indeed more effective in the treatment of cognitive and behavioural disturbances in AD, reflected in e.g. the maintenance of improvements in cognition and behaviour after cessation of stimulation.

Intensity and Pulse Form

Intensity shows considerable variation among the various studies, ranging from 65 μA - 1300 μA and 1 V - 20 V, in some indirect stimulation studies provoking muscular twitches.

Specific information on the pulse form is often lacking. The role these two stimulation-parameters can play in an optimal stimulation of the DRN and LC as an intervention strategy in AD, should be addressed in studies to be performed.

Limitations and Suggestions

In the first place, studies on indirect and direct stimulation of the DRN and LC reviewed here are no intervention studies and hence information on the most efficient stimulation-time and treatment-period is lacking. Although in our TENS-studies a stimulation-duration of 30 min a day and a treatment-period of six weeks proved to be effective (43), future studies should examine whether an extension of both parameters may be even more effective, for example by maintaining the observed effects after cessation of stimulation.

Next, the here reviewed studies are all animal experimental studies. Hence, generalization of the results to humans should take place with care. In the third place, one should be cautious when deducing stimulation-parameters for a non-invasive treatment like TENS from direct stimulation studies and invasive techniques like electro-acupuncture.

Finally, the present review does not explain *why* TENS is effective in AD. It is known that both the DRN and LC are part of the ascending reticular activating system (ARAS) that plays a central role in arousal (22). Until now it is assumed that an increase in arousal is responsible for the effects of TENS on cognition and behaviour in several conditions that effect the central nervous system (49). On the other hand, Davis and colleagues (9) found in an fMRI study increased activity in the anterior cingulate cortex, a frontal lobe area involved in attention, resulting from median nerve stimulation by TENS. These findings imply that future studies should include brain-imaging techniques that will enhance the insight into the mechanisms underlying the effects of TENS in AD.

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